Evaluation of Ration Based on Soy Sauce By-Product on Addition of Acacia and Chestnut Tannin: An *In Vitro* Study

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Evaluation of Ration Based on Soy Sauce By-Product on Addition of Acacia and Chestnut Tannin: An *In Vitro* Study

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Abstract. Feed is a main challenge for a livestock farm, especially during dry season when feed production is low. Such condition may cause low livestock productivity due to insufficient nutrient intake. Provision of high quality forages from grasses and legumes are limited by land competition for various developmental reasons. Therefore, an opportunity to provide feed for livestock is through utilization of agroindustrial by-products such as soy sauce by-product. The objective of the present study was to evaluate soy sauce by-product on addition of tannins, i.e., Acacia mangium Wild. (AT) and chestnut tannin (CT) using an *in vitro* rumen fermentation technique. The following treatments were tested: soy sauce by-product as control (R1), soy sauce by-product + 1% AT (R2), soy sauce by-product + 2% AT (R3), soy sauce by-product + 1% CT (R4) and soy sauce by-product + 2% CT (R5). The treatments were incubated *in vitro* with buffered-rumen fluid in four replicates, represented by three incubation units per replicate, and conducted for 24 h at 39 °C. Parameters measured in this research were *in vitro* gas production at regular time point intervals. Other parameters measured after the incubation were dry matter degradation, organic matter degradation, total volatile fatty acids (VFA), ammonia (NH3) and pH. Data were tested using analysis of variance (ANOVA), if there was a significant different at $P<0.05$ then continued with Duncan's multiple range test. The results of this study showed that the addition of tannins, both acacia and chestnuts, had no significant effect ($p>0.05$) to dry matter degradation, organic matter degradation, total volatile fatty acids (tVFA), NH_3 and pH. Acacia tannin was able to influence ($p<0.05$) the accumulation of gas production at 8, 12 and 24 h. It can be concluded that the addition of tannin, both acacia and chestnuts up to level 2% does not interfere with the fermentation process of soy sauce by-product in the rumen an in vitro.

1. Introduction

Soy sauce by-product has many soluble components and high degradable protein value. Its protein is not resistant to microbial degradation in the rumen and therefore needs to be protected [1] before given to livestock. Protection of feed protein can be done by either heat treatment or with the addition of formaldehyde. Such protection may increase undegradable protein fraction by approximately 50-80%. The undegraded protein in the rumen can directly undergoes enzymatic digestion process in the abomasum and small intestine. Protection of protein may also use phenolic compounds such as tannin [2], one of them can be extracted from the bark of acacia (*Acacia mangium* Wild.) [3].

Acacia is known to contain a high tannin concentration. The type of tannin present in acacia is condensed tannin or proanthocyanidin, which is a polymer of catechin and epicatechin. The other type of tannin is hydrolysable tannin. This tannin has polyester that easily hydrolyzed by acid or enzymes digestive tract to result polyphenolic acids and simple sugars [4, 5]. Tannin can be used as a feed additive to bind protein, so that the protein becomes resistant to degradation by protease either in the silo or in the rumen [6].

The objective of the present study was to evaluate soy sauce by-product on addition of tannin extract from acacia (AT) or from chestnut (CT) using an *in vitro* rumen fermentation technique.

2. Materials and methods

Soy sauce by-product was obtained from a soy sauce industry (PT Zebra, Cihideung Ilir, Ciampea, Bogor). *Acacia mangium* Wild. bark was collected from Jambi. The bark was extracted, oven-dried at 50°C for 24 h and ground finely to pass 1 mm sieve. The following treatments were tested: soy sauce by-product as control (R1), soy sauce by-product + 1% AT (R2), soy sauce by-product + 2% AT (R3), soy sauce by-product + 1% CT (R4) and soy sauce by-product + 2% CT (R5).

The *in vitro* incubation was performed by using the method of Theodorou [7]. Samples were incubated together with buffered rumen fluid at 39°C for 24 h in 4 replicates. The amounts of soy sauce by-product, rumen fluid, and buffer solution in each bottle were 500 mg DM, 17 mL, and 33 mL, respectively. Determination of gas production was performed by manual reading from the calibrated scale on the syringe. After 24 h incubation, the fermentation fluid was analyzed for pH, ammonia concentration [8], and total volatile fatty acid (VFA) [9]. Solid and supernatant parts in each incubation bottle were separated using a centrifuge at 6000 rpm for 10 second. The solid residue was subjected to analysis of dry matter degradability (DMD) and organic matter degradability (OMD) [10].

Data were subjected to analysis of variance by using a completely randomized design. Multiple comparisons among means were carried out using Duncan's test.

3. Results and discussion

Soy sauce by-product characteristics with addition acacia and chestnut tannins were not significantly different for ruminal pH, NH_3 and total VFA (Table 1).

Table 1. *In vitro* rumen fermentation profiles of soy sauce by-product with addition of tannin extract from acacia (AT) or chestnut (CT)

Treatment	pH	NH_3 (mM)	Total VFA (mM)
Soy sauce by-product	6.77 ± 0.07	19.80 ± 1.68	139.48 ± 8.92
Soy sauce by-product + 1% AT	6.74 ± 0.04	17.95 ± 3.34	128.74 ± 23.73
Soy sauce by-product + 2% AT	6.71 ± 0.02	19.51 ± 1.46	134.13 ± 17.92
Soy sauce by-product + 1% CT	6.70 ± 0.03	19.25 ± 1.96	137.20 ± 13.10
Soy sauce by-product + 2% CT	6.73 ± 0.05	15.68 ± 0.38	116.28 ± 10.29

Rumen pH in this study ranged from 6.71 to 6.77. It was within the comfortable range for rumen microbes to proliferate. Rumen pH normally range between 5.5 and 7.0, and the value can be affected by type of feedstuff and the ratio of forage to concentrate [11]. With regard to ruminal NH_3 concentration, the optimum concentration of NH_3 in the rumen is around 85 to 300 mg/L, equivalent to

6-21 mM [12], in order to sufficiently support microbial protein synthesis. Ammonia concentration in the present study was similar among the treatments. The level of protein, the solubility of protein and carbohydrate sources as well as the proportion of the solute can affect the concentration of NH_3. In addition, the concentration of NH_3 can also be influenced by the protein degradability from feedstuff, and the ability of the protein to survive from rumen microbial degradation [13].

Similar to NH_3, VFA is an end product of fermentative digestion in the rumen [14]. The VFA is the end product of carbohydrate digestion that consisted of acetate, propionate, and butyrate, with average molar ration of 65, 21, and 14%, respectively [15]. The total VFA concentration in the present study was similar among the treatments. An ideal concentration of total VFA in the rumen is about 80-160 mM [16].

The DMD and OMD values of soy sauce by-product added with either tannin extract from acacia or chestnut were similar across all treatments (Table 2).

Table 2. Dry matter degradability (DMD) and organic matter degradability (OMD) of soy sauce by-product with addition of tannin extract from acacia (AT) or chestnut (CT)

Treatment	DMD	OMD
Soy sauce by-product	33.21 ± 4.22	24.35 ± 5.11
Soy sauce by-product + 1% AT	33.46 ± 2.23	25.33 ± 8.86
Soy sauce by-product + 2% AT	28.18 ± 1.69	16.71 ± 1.25
Soy sauce by-product + 1% CT	32.28 ± 1.35	22.46 ± 1.11
Soy sauce by-product + 2% CT	34.57 ± 4.14	27.13 ± 2.23

This may indicate that AT or CT addition at 1 or 2% from dry matter does not negatively affect DMD and OMD of soy sauce by-product under *in vitro* rumen environment [17].

Cumulative gas production of soy sauce by-product with addition of tannin extract from acacia or chestnut is presented in Table 3.

Table 3. *In vitro* gas production (ml) of soy sauce by-product with addition of tannin extract from acacia (AT) or chestnut (CT)

Treatment	Gas production		
	8 h	12 h	24 h
Soy sauce by-product	13.25 ± 3.59[a]	22.75 ± 4.57[a]	36.00 ± 5.35[a]
Soy sauce by-product + 1% AT	7.75 ± 0.50[b]	16.00 ± 0.82[b]	29.00 ± 0.82[b]
Soy sauce by-product + 2% AT	8.00 ± 0.82[b]	16.00 ± 0.82[b]	28.75 ± 1.26[b]
Soy sauce by-product + 1% CT	13.50 ± 3.70[a]	21.75 ± 3.30[a]	35.00 ± 3.16[a]
Soy sauce by-product + 2% CT	13.75 ± 3.86[a]	22.00 ± 4.69[a]	34.50 ± 4.43[a]

Addition of tannin from acacia at the level of 1 and 2% decreased gas production at 8, 12 and 24 h as compared to control ($P<0.05$), whereas it was not the case for chestnut tannin addition. Apparently this is related to the different types of tannin present in both plant materials; acacia tannin has been known to be condensed tannin whereas chestnut tannin is hydrolysable tannin. The lower accumulation of gas production has to do with the effects of tannin on feed degradation in the rumen [18].

Gas production is a result of feed fermentation in rumen that depends on feed nutrient, including protein [19]. Protein content of soy sauce by-product used in the present study is high, i.e., 34.5% (dry matter basis). Mechanism of tannin in reducing gas production is through its ability to interact with feed components, mainly protein and carbohydrate, which have a major contribution in generating gas [20, 21].

4. Conclusion

The acacia tannin can be used to protect protein of soy sauce by-product in the rumen and serves as a potential feed additive for ruminant feeding.

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References

[1]. Jayanegara, A., Goel, G., Makkar, H.P.S., Becker, K. 2015a. Divergence between purified hydrolysable and condensed tannin effects on methane emission, rumen fermentation and microbial population in vitro. Anim. Feed Sci. Technol. 20960–68.

[2]. Jayanegara, A., Makkar, H.P.S., Becker, K. 2015b. Addition of Purified Tannin Sources and Polyethylene Glycol Treatment on Methane Emission and Rumen Fermentation in Vitro. Media Peternak. 38(1):57–63.

[3]. Santoso, B., Hariadi, B.Tj., Manik, H., Abubakar, H. 2011. Silage Quality of King Grass (Pennisetum purpureophoides) Treated with Epiphytic Lactic Acid Bacteria and Tannin of Acacia. Media Peternak. 34(2):140-145.

[4]. Gerlach, K., Pries, M., Südekum, K.H. 2018. Effect of condensed tannin supplementation on in vivo nutrient digestibilities and energy values of concentrates in sheep. Small Rumin. Res. 161:57–62.

[5]. Perez-Maldonado, R.A., Norton, B.W. 1996. Digestion of 14C-labelled condensed tannins from Desmodium intortum in sheep and goats. Br. J. Nutr. 76(4):501–513.

[6]. Kondo, M., Kita, K., Yokota, H. 2004. Effects of tea leaf waste of green tea , oolong tea , and black tea addition on sudangrass silage quality and in vitro gas production. 727:721–727.

[7]. Theodorou, M.K., Brook, A.E. 1990. Evaluation of a New Laboratory Procedure for Estimating the Fermentation Kinetic of Tropical Feeds. Annual Report AFRC Institute, Hurley, Maidenhead, UK.

[8]. Conway, E.J. 1950. Microdiffusion Analysis and Volumetric Error. 3rd Ed. Crosby Lokswood and Sons, Ltd., London.

[9]. Association of Official Analytical Chemist. 2012. Official Methods of Analysis, AOAC International. 19th Ed., Association of Official Analytical Chemist, Washington DC., USA.

[10]. Oliveira, S.G., Berchielli, T.T., Pedreira, M.S., Primavesi, O., Frighetto, R., Lima, M.A. 2009. Effect of tannin levels in sorghum silage and concentrate supplementation on apparent digestibility and methane emission in beef cattle. Anim. Feed Sci. Technol. 135:236–248.

[11]. Campbell, J.R., Douglas, M., Kenealy., Campbell, K.L. 2003. Animal Sciences. 4th Edition. McGraw-Hill, New York, USA.

[12]. McDonald, P.P., Edwards, R., Greenhalgh, J., Morgan, C., Sinclair, L., Wilkinson, R. 2011. Animal Nutrition. New York (USA): Prentice Hall.

[13]. Wu, G. 2017. Principles of Animal Nutrition. New York (US): Taylor & Francis Group, LLC.

[14]. Makkar, H.P.S., Blümmel, M., Becker, K. 1995. Formation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in in vitro techniques. Br. J. Nutr. 73(06):897.

[15]. Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D., Schneider, W.J. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93: 217-222.

[16]. McNabb, W.C., Waghorn, G.C., Peters, J.S., Barry, T.N. 1996. The effect of condensed tannins in Lotus pedunculatus on the solubilization and degradation of ribulose-1,5-bisphosphate carboxylase (EC 4.1.1.39/Rubisco) protein in the rumen and the sites of Rubisco digestion. Br.

J. Nutr. 76, 535–549.
[17]. Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74(10):3583–3597.
[18]. Waghorn, G.C., Ulyatt, M.J., John, A., Fisher, M.T. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on lotus. Br JNutr 57:115–126.
[19]. Getachew, G., Makkar, H.P S., Becker, K. 2001. Method of polyethylene glycol application to tannin-containing browses to improve microbial fermentation and efficiency of microbial protein synthesis from tannin-containing browses. Anim. Feed Sci. Technol. 92(1–2):51–57.
[20]. Makkar, H.P.S., Francis, G., Becker, K. 2007. Bioactivity of phytochemicals in some lesser-known plants and their effects and potential applications in livestock and aquaculture production systems. Animal 1(9):1371–1391.
[21]. Makkar, H.P.S. 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Rumin. Res. 49(3):241–256.